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Green Light Drives CO_2 Fixation Deep within Leaves [1]

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Maximal $^{14}CO_2$-fixation in spinach occurs in the middle of the palisade mesophyll [Nishio et al. (1993) *Plant Cell* 5: 953], however, ninety percent of the blue and red light is attenuated in the upper twenty percent of a spinach leaf [Cui et al. (1991) *Plant Cell Environ.* 14: 493]. In this report, we showed that green light drives $^{14}CO_2$-fixation deep within spinach leaves compared to red and blue light. Blue light caused fixation mainly in the palisade mesophyll of the leaf, whereas red light drove fixation slightly deeper into the leaf than did blue light. $^{14}CO_2$-fixation measured under green light resulted in less fixation in the upper epidermal layer (guard cells) and upper most palisade mesophyll compared to red and blue light, but led to more fixation deeper in the leaf than that caused by either red or blue light. Saturating white, red, or green light resulted in similar maximal $^{14}CO_2$-fixation rates, whereas under the highest irradiance of blue light given, carbon fixation was not saturated, but it asymptotically approached the maximal $^{14}CO_2$-fixation rates attained under the other types of light. The importance of green light in photosynthesis is discussed.

Key words: Carbon fixation gradient — Green light — Photosynthesis — *Spinacia oleracea*.

The light microenvironment within leaves must have profound effects on the distribution of carbon fixation within leaves (Gates et al. 1965, Nishio et al. 1993, Osborne and Raven 1986), as the light intensity, as well as quality, is highly variable (Vogelmann 1993). Generally, red and blue light are strongly attenuated in the upper part of the leaf, whereas green and far-red light are transmitted more deeply into the leaf (Cui et al. 1991, Gates et al. 1965, Strain 1950, Terashima and Saeki 1985, Vogelmann 1993).

We recently showed that in spinach leaves, CO_2 fixation is disconnected from the light gradients across leaves (Nishio et al. 1993). The maximum rates of carbon fixation under white light occur deep within the leaf (mid-palisade and lower) in bean (Jeje and Zimmermann 1983) and spinach (Nishio et al. 1993); not at the top of the leaf (upper epidermis plus upper 20–25% of the palisade mesophyll, also referred to as the upper part of the leaf), where the greatest intensity of light is expected (Cui et al. 1991, Terashima and Saeki 1983, Vogelmann 1993). These findings suggest the possibility that maximal light absorption occurs in the middle layers of the mesophyll rather than in those cell layers nearest the adaxial leaf surface (Evans 1995), although it has been shown that a steep light gradient exists across spinach leaves (Cui et al. 1991).

Chloroplast adaptation to sun and shade conditions within leaves is well-documented (Nishio et al. 1993, Outlaw 1987, Terashima 1989). However, the role of different quality light at different depths within leaves has not been established. Since blue and red light are rapidly attenuated across leaves, and green light penetrates more deeply into leaves, we tested the role of different light quality on CO_2 fixation profiles across spinach leaves using broad-band red, green, and blue light of different irradiance. We showed that green light, which Chl does not absorb as strongly as red or blue light, drives CO_2 fixation deeper within leaves than does red or blue light, and that blue and red light contribute to most of the fixation that occurs in the upper part of spinach leaves. The pigment complement utilized by higher plants, as well as variations in leaf thickness and pigment distribution within the leaf due to growth conditions, allows dynamic utilization of light within the leaf.

Materials and Methods

Plant growth conditions—Spinach (*Spinacia oleracea* cv. hybrid 424, Ferry-Morse Seed Company, Modesto, CA, U.S.A.) was cultured hydroponically in 0.5 × Hoagland solution (Hoagland and Arnon 1950) in controlled environmental growth chambers under 800 μmol quanta (400–700 nm) $m^{-2}\,s^{-1}$ as previously described (Nishio et al. 1993). The temperature was 23 ± 2°C in the light and 17 ± 2°C in the dark, and the light period was 12 h. Five- to six-week old plants were used in the experiments. The average thickness of leaves was 680 μm. Cabbage (*Brassica oleracea* L., F1 hybrid Savoy King (Sakata Seed America Inc., Morgan Hill, CA, U.S.A.)), utilized for light saturation curves, was grown in the greenhouse at the University of Wyoming, Laramie, WY, U.S.A. The photoperiod was 16 h (supplemented with 1,000 watt metal arc lamps), the temperature was 28 ± 3°C, and the relative humidity was not controlled.

$^{14}CO_2$ fixation—Gradients of $^{14}CO_2$ fixation across leaves were measured as previously described (Nishio et al. 1993), except that 700 ppm CO_2 containing 1.48×10^4 Bq $^{14}CO_2$ (specific activity of 18.5×10^{10} Bq mol^{-1}) was used. A leaf was placed under assay light for 5 min, and then clamped in a small leaf chamber (1.27 cm diameter) that allowed top illumination and gas exchange through small ports on the bottom. The leaf in the chamber was illuminat-

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ed by light from a 1,000 watt xenon arc lamp directed with a fiber optic cable (1.5 cm diameter). The light was filtered to provide the monochromatic light as described below. $^{14}CO_2$ was injected into the chamber, and the leaf labeled for 5 s and chased for 5 s (time before freezing). A longer pulse and/or longer chase did not alter the patterns of fixation across the leaves. Then, a flat leaf plug was obtained from the center of the labeled leaf tissue using a frozen (in $N_2(l)$) paper punch with a copper block attached to the bottom, that flattened the leaf plug immediately prior to freezing and "punching". The resulting flat, frozen leaf plug was stored in $N_2(l)$ until it was transferred to a freezing microtome and sectioned paradermally in 40 μm increments. Chlorophyll was bleached with acetic acid before $^{14}CO_2$ incorporation was measured by liquid scintillation counting with quench correction (TriCarb 4430; United Technologies Packard, Downers Grove, IL, U.S.A.) (Nishio et al. 1993).

Photosynthetic CO_2 gas exchange—Gas exchange was measured with a CIRAS-1 (PP Systems, Bedford, MA) portable gas exchange system.

Monochromatic light—Light from a 1,000 watt xenon arc lamp was focused and directed onto a fiber optic cable that illuminated the leaf. Broad band single-color (monochromatic) light was made by inserting various light filters between the arc lamp and the fiber optic cable. Transmission spectra of the filters were scanned in an HP 8452A Diode Array Spectrophotometer (Hewlett-Packard, Waldbronn, Germany) or a Lambda 4B Spectrophotometer (Perkin-Elmer Corp., Norwalk, CT, U.S.A.). Spectral irradiance of the filters and xenon source were measured with an Optronics 742 spectroradiometer (see below). Blue light for $^{14}CO_2$ fixation was obtained with two blue filters (Kopp 4-96 and Hoya B370, λ_{max}=411 nm, half-band width=54 nm). Blue light for gas exchange was obtained with one blue filter (Manostat, Filter #42, New York, NY, U.SA., λ_{max}=411 nm, half-band width=66 nm). The wider band width allowed us to obtain higher photon fluxes. The UV light that passed through the filters was mostly eliminated by glass. Green light was obtained using one green filter (OCLI green dichroic filter, Optical Coating Laboratory, Inc., Santa Rosa, CA, U.S.A., λ_{max}=544 nm, half-band width=70 nm). Red light for $^{14}CO_2$ fixation was generated using a 611 nm cut-off filter, and red light for gas exchange was obtained using a 648 nm cut-off filter (Kopp, CS 2-64, Pittsburgh, PA, U.S.A.).

Photon flux through the filters for gas exchange and carbon fixation was measured with a LI-COR LI-185B using a quantum sensor 190SB (LI-COR, Lincoln, NE, U.S.A.), whose sensitivity to the monochromatic light was calibrated against curves for spectral irradiance of the monochromatic light. Spectral irradiance was measured with a spectroradiometer (Model 742, Optronics Laboratories, Orlando, FL, U.S.A.), that was calibrated against a 1,000 W tungsten-filament quartz-halogen standard lamp (Optronics Laboratories, traceable to a standard at the National Institute of Standards and Technology, Gaithersburg, MD, U.S.A.).

The $^{14}CO_2$ measurements were based on incident light, whereas, the quantum efficiency measurements of CO_2 gas exchange were based on absorbed light. Light absorption by leaves was measured as described previously (Gorton and Vogelmann 1996). Briefly, measurements of leaf reflectance (R) and transmittance (T) were made using an integrating sphere, 10 cm in diameter, coated with Kodak white reflectance coating (Eastman Kodak Co., Rochester, NY, U.S.A.) and coupled to a LI-1800 spectroradiometer (Li-Cor Inc., Lincoln, NE, U.S.A.) with a fiber optic cable. Collimated light was provided by a 150 W xenon-arc lamp (Bausch and Lomb, Ocean Springs, MS, U.S.A.) stabilized by an uninterruptible power source (model 450, American Power Conversion, West Kingston, RI, U.S.A.). Measurements of R and T were made from leaf discs (1 cm diameter) by scanning from 400–700 nm. Absorbancy (A) was calculated as A=1−(R+T). The measured fraction of absorbed incident light for spinach was 0.98 for blue light, 0.93 for red light, 0.91 for white light, and 0.81 for green light. For cabbage, the fraction of incident light absorbed was 0.956 for blue, 0.79 for green, 0.916 for red, and 0.89 for white light.

The difference between the filter sets used for gas exchange and carbon fixation had little impact on our analysis. The percentage of incident light absorbed by the leaf at wavelengths across the blue region of the spectrum was very flat (98–97% between 400–495 nm). Whereas, the average absorption by the leaf within the wavelength range of the 611 cut-off filter was 92.3%, and it was 93.5% for the 648 nm cut-off filter.

Results

Monochromatic red, blue, or green light caused different patterns of carbon fixation across leaves (Fig. 1). Blue light caused fixation mainly in the most upper portion of the leaves, whereas fixation under green light, was shifted more deeply into the leaves. Red light caused a fixation pattern intermediate to those under blue or green light. Different irradiances of each light type always caused the same relative pattern of fixation across the leaf (Fig. 1).

Carbon fixation across leaves, measured under blue light, increased from the upper epidermis to a maximum about 150 μm deep within the leaf (Fig. 1A). The amount of fixation deeper in the leaf dropped dramatically at depths greater than 200 μm. It appears that on an absolute basis, fixation per paradermal leaf section under blue light (Fig. 1D) was higher at the top of the leaf compared to that caused by red or green light (Fig. 1E, F). Total fixation (sum of fixation of all leaf sections across the leaf) driven by 500 μmol blue light m^{-2} s^{-1} (6,700 dpm (leaf plug)$^{-1}$), however, was lower than that under comparable red (7,000 dpm (leaf plug)$^{-1}$) or green light (7,500 dpm (leaf plug)$^{-1}$).

When $^{14}CO_2$-fixation was measured under green light, carbon fixation increased to a maximum approximately 250–300 μm below the adaxial leaf surface, then decreased with greater leaf depth. The absolute values of the slopes of the increasing and decreasing gradients were similar so a relatively gaussian distribution of fixation occurred under green light (Fig. 1B, E). The maximum fixation observed under red light was about 150–200 μm below the adaxial leaf surface (Fig. 1C, F); and the major decrease in fixation did not occur until depths greater than 300 μm. Based on the gas exchange data (see below), carbon fixation was not saturated at 500 μmol m^{-2} s^{-1}; however, the relative pattern of fixation was similar at all levels of irradiance given (Fig. 1A–C).

While maximum fixation under all light types occurred in the middle of the palisade mesophyll and not at the top of the leaf, the slopes of the ascending and descending por-

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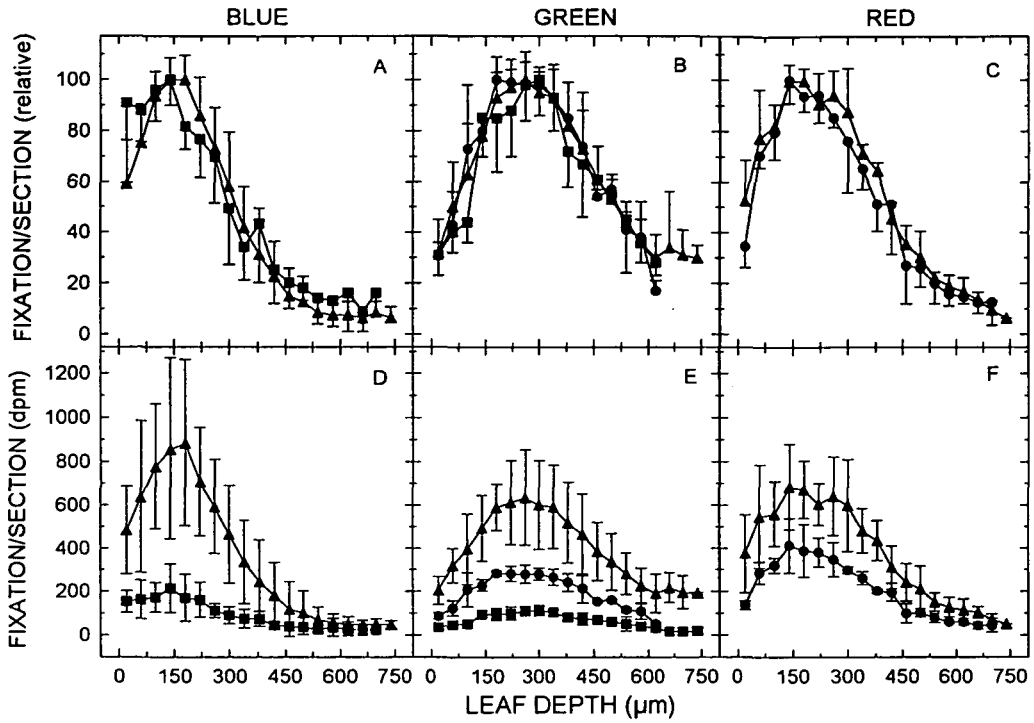

Fig. 1 Effect of different monochromatic light on carbon fixation per section across sun leaves of spinach. Carbon fixation across leaves expressed on a relative basis with maximum fixation/section set to 100 (A, B, and C), or as dpm/section (D, E, and F). Irradiances utilized were 500 μmol m^{-2} s^{-1} (▲), 200 μmol m^{-2} s^{-1} (●), and 50 μmol m^{-2} s^{-1} (■). A and D. Blue light, (▲, n=10); (■, n=8). B and E. Green light, (▲, n=10); (●, n=4); (■, n=4). C and F. Red light, (▲, n=3); (●, n=3).

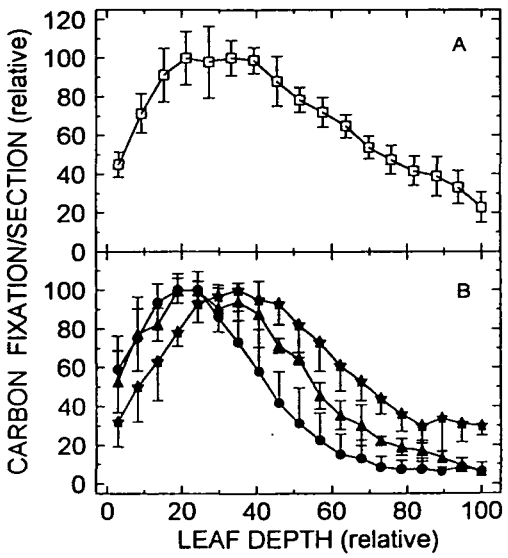

Fig. 2 Relative carbon fixation per section across sun leaves of spinach under different monochromatic light. The maximum rate per section was set at 100% under each light type. A. White light 800 μmol m^{-2} s^{-1} (□, n=11). B. Blue light, 500 μmol m^{-2} s^{-1} (●, n=10); Red light, 500 μmol m^{-2} s^{-1} (▲, n=3); Green light, 500 μmol m^{-2} s^{-1} (★, n=10).

tions of the fixation profiles and the absolute depth of maximum fixation varied with the color of light (Fig. 2). The overall shape of the fixation profile under white light (Fig. 2A) was similar to the shape obtained by summing the individual fixation patterns under blue, red, and green light (not shown). The maximum peaks of fixation under blue and red light were closer to the top of the leaf (at about 20% leaf depth) compared to that under green light (maximum about 35–40% of leaf depth) (Fig. 2). The descending leg (abaxial side) of the pattern of fixation under blue light was much steeper and not as deep into the leaf compared to the pattern of carbon fixation caused by red light (Fig. 2B). While the peak of carbon fixation under green light was deeper in the leaf than that under red or blue light, the fixation under green light at the top of the leaf was low compared to that due to red or blue light (Fig. 2B).

In spinach and cabbage, carbon fixation under white, red, or green light exhibited similar saturation levels (Fig. 3). Photosynthetic CO_2 gas exchange under blue light was significantly lower than under red, green, or white light at similar photon fluxes; however, extrapolation of the rate of gas exchange under blue light suggests that the maximum rate attained under white light could be reached with higher fluxes of blue light than we utilized. In spinach, red

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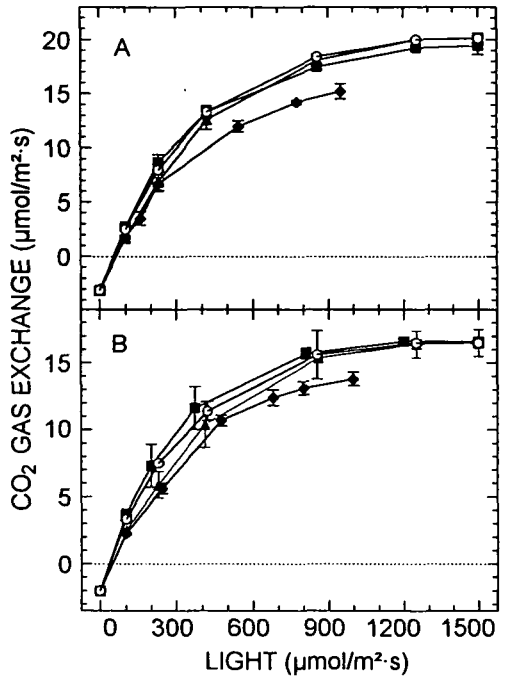

Fig. 3 Effect of wide band monochromatic light on CO_2 gas exchange rates. A. Cabbage. White (○, n=4); Blue (◆, n=4); Red (■, n=4); Green (▲, n=3). B. Sun spinach. White (○, n=2); Blue (◆, n=2); Red (■, n=2); Green (▲, n=2).

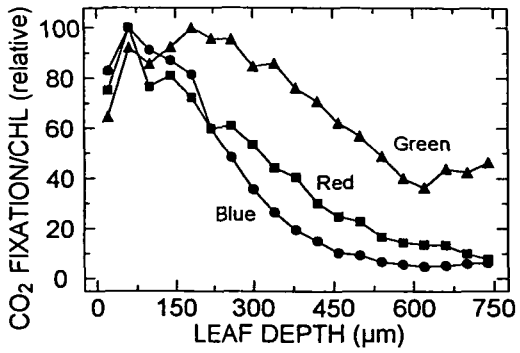

Fig. 4 Effect of monochromatic light on relative $^{14}CO_2$ fixation per Chl across spinach leaves. The photon flux of each light type was 500 μmol m^{-2} s^{-1}. Green light, n=10, (▲); Red light, n=3, (■); Blue light, n=10, (●).

light exhibited the highest quantum efficiency; whereas in cabbage, red and white light had similar quantum efficiencies (Table 1). In spinach, the quantum efficiency under green light was about 10% lower than that for red light, and for blue light it was about 25% lower compared to red light. In cabbage, the quantum efficiency for blue light was 20% lower than that for red light.

Fig. 4 shows the relative carbon fixation per Chl under the different colored lights. Plotting the data in this fashion accentuates the role of green light in driving carbon fixation more deeply and broadly across the leaf than either blue or red light. On a Chl basis, the peak of fixation by green light is about 200 μm into the leaf, whereas fixation under blue light and red light on a Chl basis peaks just below the epidermal layer. Carbon fixation under blue light decreased towards the abaxial leaf surface more rapidly compared to that under red light, and fixation per Chl is reduced to 10% of the maximum about 450 μm below the adaxial leaf surface. On a relative basis, carbon fixation per Chl under green light decreases to a minimum of about 40% of the maximal rate only at the bottom of the leaf.

Discussion

Green light is effectively absorbed by green leaves (Björkman 1968, Gabrielsen 1948, Gates et al. 1965, Kleshnin and Shulgin 1959, Monteith 1965, Moss and Loomis 1952, Rabideau et al. 1946, Seybold and Weisweiler 1943, Shibata et al. 1954, Stoy 1955, Strain 1951), and green light efficiently drives electron transport (Ghi-

Table 1 Quantum efficiency (ϕ) and yield of CO_2 uptake of intact leaves under monochromatic illumination

Species	Light quality	Initial slope (ϕ)	Quanta/CO_2 uptake
Spinach (n=6)	Red	0.062±0.001	16.1
	White	0.059±0.001	16.9
	Green	0.057±0.003	17.5
	Blue	0.047±0.002	21.3
Cabbage (n=4)	Red	0.064±0.002	15.6
	White	0.062±0.002	16.1
	Green	0.058±0.002	17.2
	Blue	0.052±0.003	19.2

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rardi and Melis 1984). Action spectra show that green light is an effective spectral region in powering photosynthesis in higher plants, especially in leaves with high Chl content (Bulley et al. 1969, Clark and Lister 1975, Inada 1976, Lundegårdh 1966, McCree 1972, Pickett and Myers 1966, Yabuki and Ko 1973). Green light is also active in the production of Chl (Sayre 1928). Because many early action spectra were determined with dilute algal suspensions, the action of red and blue light has been accentuated (e.g., Haxo and Blinks 1950). As a result, it is often assumed that green light is not important in driving photosynthesis because of the low extinction of Chl at green wavelengths compared to red and blue light (e.g., Campbell 1996, Raven and Johnson 1996).

Action spectra of higher plants (Clark and Lister 1975, Evans 1987, Inada 1976, McCree 1972) also show that the quantum efficiency of carbon fixation is highest under red light, and generally, blue light has a lower quantum efficiency than green light, even though the Chl extinction is higher in blue light. The lower quantum efficiency under blue light is due in part to blue light absorbing flavonoids, which are not in the chloroplast and cannot transfer energy to the reaction centers, and carotenoids, which may not efficiently transfer energy to the reaction centers under certain conditions.

Light saturation curves with red and green light caused equal rates of photosynthesis in *Sinapsis alba*, and the rates approached saturation under the maximum blue light utilized (Gabrielsen 1940). We also were unable to reach saturation of photosynthesis under blue light, as we were unable to produce fluxes of blue light greater than 1,000 μmol m^{-2} s^{-1}. However, our data (Fig. 3B) corroborate the findings of Gabrielson (1940). The lower rates of photosynthesis under blue light at comparable fluxes of the other types of light are due, in part, to a combination of carotenoid and flavonoid absorption. Since greater fixation per section occurred at the top of the leaf when illuminated with blue light compared to red or green light (Fig. 1D, E, F), it is likely that more blue light energy was absorbed and transferred to the reaction centers in the upper portion of the leaf. Thus, it is also possible that induction of non-photochemical quenching and photoinhibition caused the decreased rates of photosynthesis under blue light, especially at wavelengths between 380–430 nm (Jones and Kok 1966), which we used (see materials and methods).

We showed that green light drives photosynthetic carbon fixation deeper within leaves than does blue or red light. In leaves with high Chl concentrations, the action in the green region of the electromagnetic spectrum is high, as the relative absorption of green light compared to red or blue light increases as the Chl concentration increases (Strain 1950, 1951). The higher contribution of blue and red light to carbon fixation at the top of the leaf and green light to fixation deeper within the leaf is due to the wavelength dependent transmission and absorption of light by the photosynthetic pigments.

Although "sun" leaves from spinach plants are significantly thicker than shade leaves, on a relative basis, the two types of leaves exhibit identical carbon fixation profiles (Sun et al. 1996) and light gradients (Cui et al. 1991). The distribution of Chl across the leaf greatly affects the light microenvironment within spinach leaves, so the Chl concentration in the upper part of a thick leaf must be lower than in the upper region of a thin leaf for similar light distribution to occur in both leaf types. Indeed, Chl per volume (paradermal leaf section) is lower in the upper portion of sun spinach leaves than in shade spinach leaves (Nishio et al. 1993). Also, the epidermis contains little Chl because only guard cells contain Chl, and the maximum Chl content per volume is in the middle of the leaf.

It appears that mesophytic leaves, such as spinach leaves, have an "optimal" leaf that exhibits photosynthetic gradients that are dependent on both the distribution of photosynthetic capacity across the leaf and leaf anatomy. There is a strong correlation between carbon fixation and Rubisco, but not between Chl and carbon fixation (Nishio et al. 1993). Why maximum carbon fixation (Rubisco) occurs in the middle of the palisade mesophyll and not at the top of the leaf, however, has not yet been clearly explained.

Under low photon fluxes of monochromatic light, the pattern of fixation across the leaves was the same as under high photon fluxes (Fig. 1). It was not possible to significantly shift the relative amount of fixation towards the top of the leaf by illumination with a photon flux of blue light as low as 50 μmol m^{-2} s^{-1} (compared to fixation under 500 μmol blue light m^{-2} s^{-1}). However, the relative fixation in the uppermost section under blue light of 50 μmol m^{-2} s^{-1} was higher than under 500 μmol m^{-2} s^{-1}. This large difference was not seen under red or green light, so it raises the possibility that the epidermal section was photoinhibited by 500 μmol blue light m^{-2} s^{-1}. Such a shift was not detectable when leaves were photoinhibited with white light, but we did not measure carbon fixation under 50 μmol white light m^{-2} s^{-1} (Sun et al. 1996).

Similar rates of CO_2 gas exchange under low levels of blue and green light were measured (the rate under green light is slightly higher). While the quantum efficiency of absorbed green and blue light are somewhat similar, the utilization of incident light is much lower for green light than for blue because the fraction of light absorbed is lower for green light by about 20% in spinach leaves. Thus, at low light intensities, both red and blue light drive more photosynthesis than does green light because relatively more blue and red light are absorbed and used to drive electron transport (compare 1D, E).

It appears that on an absolute basis, fixation under blue light was higher at the top of the leaf compared to that caused by red or green light (Fig. 1D–F). Based on the ab-

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sorption data, fixation under blue light should be 7% higher than fixation under red light and 17% higher than under green light (if all light was absorbed by Chl). However, the total fixation under 500 μmol blue light $m^{-2} s^{-1}$ was 6,700 dpm (leaf plug)$^{-1}$ (90% of green light), under a similar flux of red light the total fixation was 7,000 dpm (leaf plug)$^{-1}$ (94% of green light), and under green light it was 7,500 dpm (leaf plug)$^{-1}$ (100%) (Fig. 1). CO_2 gas exchange under 500 μmol blue light $m^{-2} s^{-1}$ was also slightly lower than under the other types of light (Fig. 3B). Gas exchange under 500 μmol red light $m^{-2} s^{-1}$ was about 15% higher than under an equivalent flux of green light. Thus, photosynthesis under red and green light fluxes of 500 μmol $m^{-2} s^{-1}$ were about the same, whereas under blue light, fixation was lower by about 7-10%.

Since fixation under blue light occurred mainly in the upper portion of the leaf and the pattern of fixation is relatively fixed, we concluded that more fixation per section does indeed occur in the upper part of the leaf under blue light than under green or red light (Fig. 1D, E, F), although the standard deviation of the absolute data for blue light was large. Red light also caused more fixation at the top of the leaf than green light, but not to the same extent as blue light. Though unlikely, it is also possible that the difference in absolute fixation may be due to differences in leaf samples rather than in light quality.

Contrary to thinking that green light is the least utilized photosynthetically active radiation as often stated in biology text books, our data and that of earlier researchers (e.g., Bulley et al. 1969, Clark and Lister 1975, Inada 1976, Lundegårdh 1966, McCree 1972, Pickett and Myers 1966, Strain 1951, Yabuki and Ko 1973) highlights that green light is a significant energy source in driving photosynthesis, particularly at the whole plant level. The complement of Chl $a+b$ and carotenoids that evolved in algae and is utilized by higher plants allows efficient utilization of the broad spectrum of visible light that impinges on plants. Although green light provides the greatest amount of radiant energy that reaches the earth's surface (see Kirk 1994), Chl *a* and Chl *b* exhibit their lowest extinction in the green, which allows green light to be transmitted deeply within leaves. While red light clearly has the highest quantum efficiency and is the most efficiently utilized light, it is mainly absorbed in the upper portion of leaves. Based on the carbon fixation rates (Fig. 1), however, more blue light is absorbed at the top of the leaf. Since blue light is also absorbed by flavonoids and carotenoids, blue light has a lower quantum efficiency than red light does. Green light, on the other hand, has a quantum efficiency higher or equivalent to that of blue light, and it is transmitted through the leaf and to underlying leaves where it can actively drive photosynthesis (Fig. 4).

Thus, the low extinction of green light by Chl allows transmission of light required for photosynthesis that can be absorbed deeper within the leaf. In high light, leaves often are thicker, and the absorption of green light is increased because the leaves have a higher concentration of Chl per leaf area. Green light absorption is also increased by light scattering. In the shade, the leaves are thinner, but the Chl concentration on a weight or volume basis may be higher (Nishio et al. 1993). By varying the concentration of Chl, plants can modulate the amount of green light that is absorbed, as most of the red and blue light is absorbed even in leaves with low Chl content (Moss and Loomis 1952, Rabideau et al. 1946, Strain 1951, Yabuki and Ko 1973).

Leave possess mechanisms to deal with light absorbed in excess of that which can be utilized by the electron transport chain (Demmig-Adams and Adams 1992). Absorption of light by non-photosynthetic pigments decreases photosynthetic efficiency. Conifers in particular exhibit decreased action in the blue region of the visible spectrum (Burns 1942). Carotenoids and flavonoids have a role in the decreased efficiency of blue light in driving photosynthesis (Clark and Lister 1975, Gabrielsen 1948, Inada 1976). With regard to non-photochemical quenching, carotenoids may be directly involved in dissipation of absorbed light energy by plants (Demmig-Adams and Adams 1992, Horton and Ruban 1994, Niyogi et al. 1997, Owens 1994). Anthocyanins can also decrease the efficiency of absorbed light for photosynthesis, however many higher plants do not have significant quantities of anthocyanin during the majority of the growing season.

Excitation of Chl at the top of the leaf by blue and red light is higher than with green light. The high extinction of blue and red light suggests the possibility that the top of the leaf has a greater capacity for non-photochemical quenching than underlying tissue. Under full sun, where light is 3-5 times saturation for many plants, nonphotochemical quenching could be engaged at the top of the leaf, but deeper within the leaf, where mainly green light is absorbed, nonphotochemical quenching would not be active. Such separation of "efficient" and "down-regulated" photosynthesis across the leaf would protect the upper cell layers of the leaf from photoinhibition (Sun et al. 1996), yet at the same time allow carbon fixation to effectively continue even under greater than saturating light.

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